Methods for Increasing the Expression Level of a Soluble Fusion Protein Encoding a 62 Amino Acid Fragment of the α-Subunit of the Nicotinic Acetylcholine Receptor[a]

LISA N. GENTILE AND EDWARD HAWROT

Section of Molecular and Biochemical Pharmacology
Brown University
Providence, Rhode Island 02912

We are interested in expressing a large soluble fragment of the α-bungarotoxin (BGTX) binding domain of the nicotinic acetylcholine receptor (nAChR) to facilitate structural studies by high-resolution NMR spectroscopy.[1] As such, we need to express the receptor fragment (with and without the possibility for metabolic ^{15}N and/or ^{13}C labeling) in multimilligram quantities. Toward this end we have constructed a synthetic gene from overlapping oligonucleotides of a T62mer (*Torpedo californica* α-subunit residues 143–204: TMKLGIWTYDGTKVSISPESDRPDLST-FMESGEWVMKDYRGWKHWVYYTCCPDTPYLDITYH). The T62mer receptor gene was appended to the C-terminus of gene 9 (in plasmid pSR9), a highly soluble bacteriophage *T7* coat protein, separated by an acid cleavage (DP) site. The expression of the fusion protein is cytoplasmic in *Escherichia coli*, and under the inducible control of the *lacUV5* promoter of *T7* RNA polymerase (see FIG. 1; a similar approach was used to construct and express a synthetic gene for α-BGTX).[2] Gene 9 was chosen as a fusion partner for our receptor fragment for a number of reasons: its solubility, its lack of Cys residues that could interfere with the oxidation of Cys 192–193 of the receptor, and its high levels of expression (up to 50% of the soluble protein) in other systems.[3] At present, we are able to obtain gram quantities of the fusion protein in rich media (LB) and 500–600 mg in minimal media (M9) per 2-L fermenter preparation.

MATERIALS AND METHODS

A typical protocol for harvesting the total soluble cytoplasmic proteins after induction includes centrifugation to pellet the cells, washing of the cell pellet in PBS followed by resuspension of the cells in lysis buffer, french press lysis at 2100 psi, high-speed centrifugation to collect soluble fractions (note, the protein is not found in inclusion bodies in the pellet), DEAE column to remove nucleic acids, and

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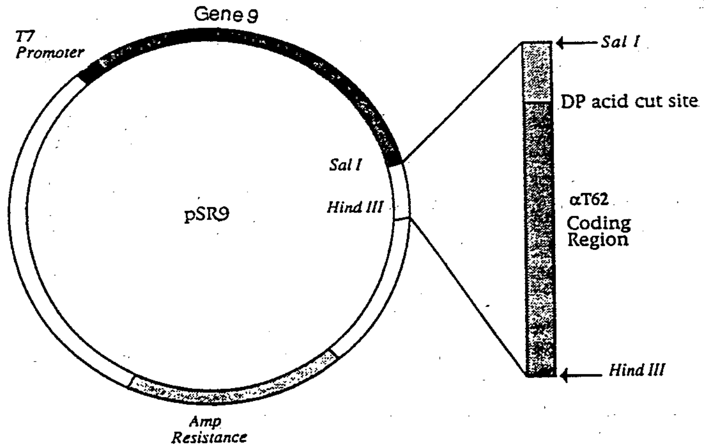

FIGURE 1. The synthetic gene 9/αT62 fusion protein. The DNA sequence for αT62 was derived from back-translation of the primary amino acid sequence, and optimized for codon usage in *Escherichia coli.* A DP acid cut site was engineered in at the 5′ end of the T62mer coding sequence to allow later liberation of the 62mer from gene 9.

ammonium sulfate precipitation. Protease inhibitors are present throughout this procedure.

RESULTS AND DISCUSSION

To ensure that we were expressing as much fusion protein as possible for our system, we performed protein expression checks of the cultures derived from a number of different BL21(DE3) isolates containing fusion protein insert. We found, but cannot explain, the presence of both "high" and "low" expressing colonies from a single transformation of our fusion protein expressing plasmid into our host strain. Growth experiments were performed under various conditions to maximize the final OD_{600} of the culture. The results of these studies are shown in TABLE 1 below. The greatest yields of fusion protein were obtained when the cultures were grown in a 2-L bench-top fermenter, in LB media supplemented with glucose.[4] Under these condi-

TABLE 1. Comparison of Cell Growth in Various Media

Prep Conditions (37 °C)	Medium	OD_{600} at Induction	Final Uninduced OD_{600}	Induced Protein
2L/Fernbach	LB	0.7	1.4	350 mg
2L/Fermenter	LB	2.4	4.3	470 mg
2L/Fermenter	LB + 4 g glucose	4.7	8.5	1080 mg
2L/Fermenter	M9	1.6	3.7	N/A
2L/Fermenter	M9 + 7.2 g glucose spike at OD_{600} ~ 1	2.3	4.8	540 mg

tions, and with the IPTG induction at mid-log phase, we harvested >1 g of total protein at the ammonium sulfate precipitation step. We estimate that approximately one-third of this is our induced fusion protein as judged by SDS-PAGE (further purification can be achieved by FPLC on a Mono-Q column).

As we are interested in ^{15}N and/or ^{13}C metabolic labeling of our receptor fragment for NMR studies, it is important that we can express high levels of the fusion protein in a defined medium. From 2-L cultures grown in M9 medium, where NH_4Cl is the only source of nitrogen and either glucose or NaOAC are the only sources of carbon, we obtained >0.5 g of our fusion protein. Although it may be possible to increase the levels of expression even further by monitoring the glucose level in the fermenter and supplying the bacteria with increased O_2, the inexpensive modifications made above should enable us to produce the materials needed for structural studies.

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